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13. Abstract (Maximum 200 Words) <i>(abstract should contain no proprietary or confidential information)</i>  Recently it was shown that the lysophosphatidic acid (LPA) receptor LPA <sub>3</sub> /EDG7 is expressed in human prostate cancer cell lines namely LNCap and Du-145. Based on these findings and evidence that LPA is a very potent mitogen, the overall goal of this proposed research is to rationally design LPA <sub>3</sub> /EDG7 selective ligands. We have identified the probable binding position of two LPA <sub>1</sub> /EDG2 and LPA <sub>3</sub> /EDG7 selective inhibitors namely DGPP (8:0) and FAP (12:0). The binding position and the critical amino acids involved in interacting with these inhibitors relative to the endogenous ligand LPA (18:1) is discussed.			
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## **Table of Contents**

<b>Cover.....</b>	<b>1</b>
<b>SF 298.....</b>	<b>2</b>
<b>Introduction.....</b>	<b>4</b>
<b>Body.....</b>	<b>4</b>
<b>Key Research Accomplishments.....</b>	<b>9</b>
<b>Reportable Outcomes.....</b>	<b>10</b>
<b>Conclusions.....</b>	<b>10</b>
<b>References.....</b>	<b>11</b>
<b>Appendices.....</b>	<b>13-23</b>

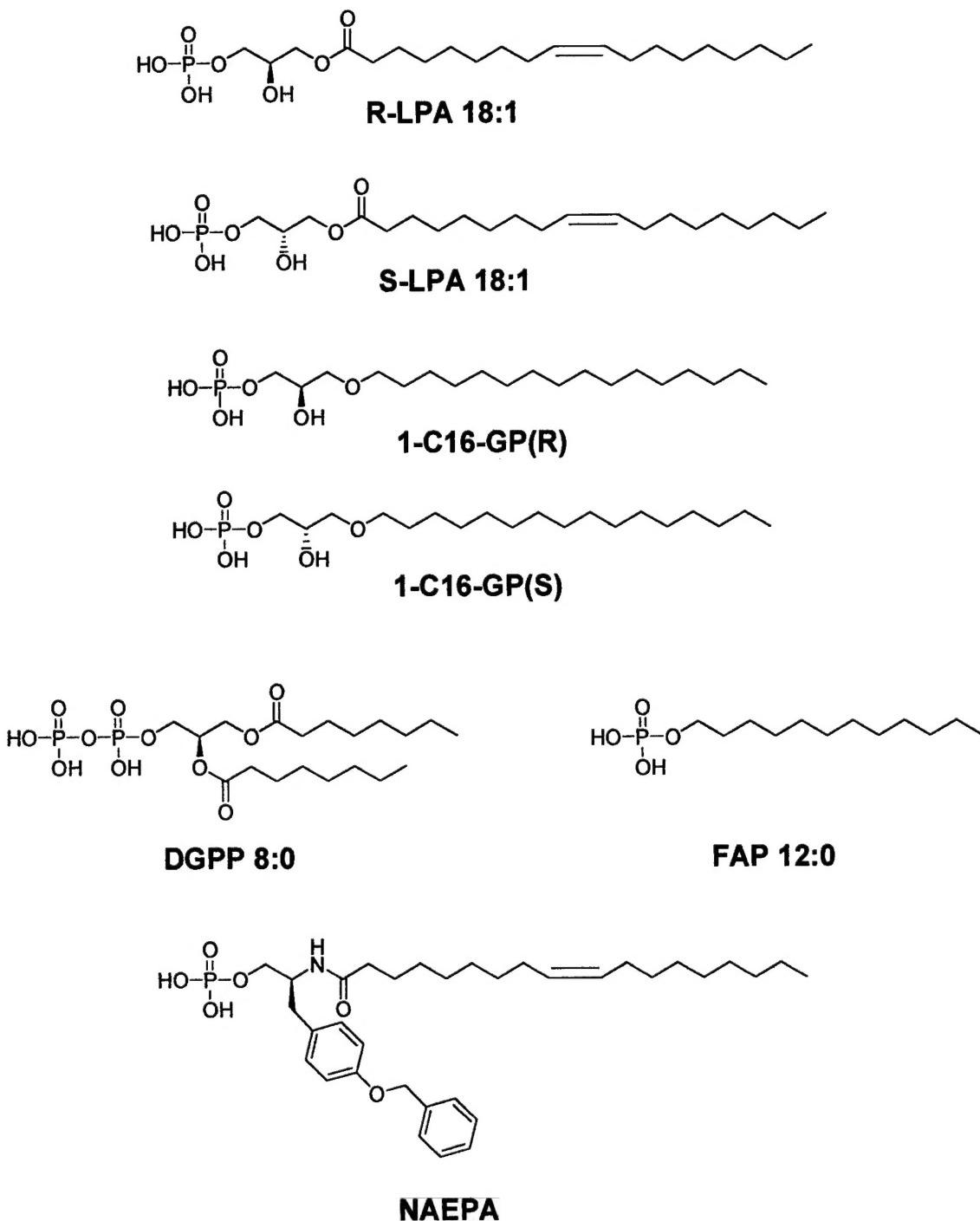
## Introduction

Lysophosphatidic acid (LPA) elicits a variety of responses including mitogenesis,<sup>1-3</sup> cytoskeletal changes,<sup>4-10</sup> activation of Ca<sup>2+</sup> transients,<sup>11-13</sup> and effects on apoptosis.<sup>14</sup> These responses are elicited via the LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4, and LPA<sub>3</sub>/EDG7 G protein-coupled receptors. These receptors are members of the endothelial differentiation gene family. In order to understand the physiological significance of LPA highly selective antagonists are necessary.

The work of Imamura and colleagues has established that cancer cells require serum factors for invasion<sup>15</sup> and that LPA is the major serum factor responsible for this effect. This invasive effect is mediated by the Rho signaling pathway. LPA is fully capable of restoring tumor cell invasion in serum free systems<sup>10,16</sup>. The central goal of the present study is to develop and validate an LPA<sub>3</sub>/EDG7 model and based on this model rationally design ligands with receptor subtype-specific action, that will provide critically needed new tools and knowledge in this developing field. The development of validated computational receptor models will aid future attempts to develop such compounds, which may be useful in understanding and eventually treating prostate cancer.

## BODY

Three antagonists selective for LPA<sub>1</sub> and LPA<sub>3</sub> have recently been identified. First, DGPP (8:0) (**Figure 1**) was shown to be a selective inhibitor of LPA<sub>1</sub> and LPA<sub>3</sub>. DGPP inhibited LPA-induced Ca<sup>2+</sup> transients in stably-transfected RH7777 cells expressing LPA<sub>1</sub> and LPA<sub>3</sub> with K<sub>i</sub> of 6600±680 and 106±28 nM respectively.<sup>17</sup> However DGPP had no effect on LPA-induced Ca<sup>2+</sup> response in stably-transfected RH7777 cells expressing LPA<sub>2</sub>. Second compound, FAP (12:0) (**Figure 1**) was found to be a selective antagonist of LPA<sub>1</sub> and LPA<sub>3</sub>. FAP inhibited LPA-induced Ca<sup>2+</sup> responses in RH7777 cell expressing LPA<sub>1</sub> and LPA<sub>3</sub> with a K<sub>i</sub> values of 9 μM and 89 nM respectively. FAP did not inhibit LPA-induced Ca<sup>2+</sup> mobilization in RH7777 cells expressing LPA<sub>2</sub>, however FAP activated Ca<sup>2+</sup> mobilization in these cells with an EC<sub>50</sub> 700 nM<sup>18</sup>. Finally NAEPA (**Figure 1**) was also shown to be a selective antagonist of LPA<sub>1</sub> and LPA<sub>3</sub>. NAEPA inhibited LPA induced GTP [ $\gamma^{35}$ S] binding in RH7777 cells expressing LPA<sub>1</sub> and LPA<sub>3</sub> with K<sub>i</sub> of 137 nM and 428 nM respectively.<sup>19</sup>



**Figure1:** Agonist and antagonist of LPA receptors

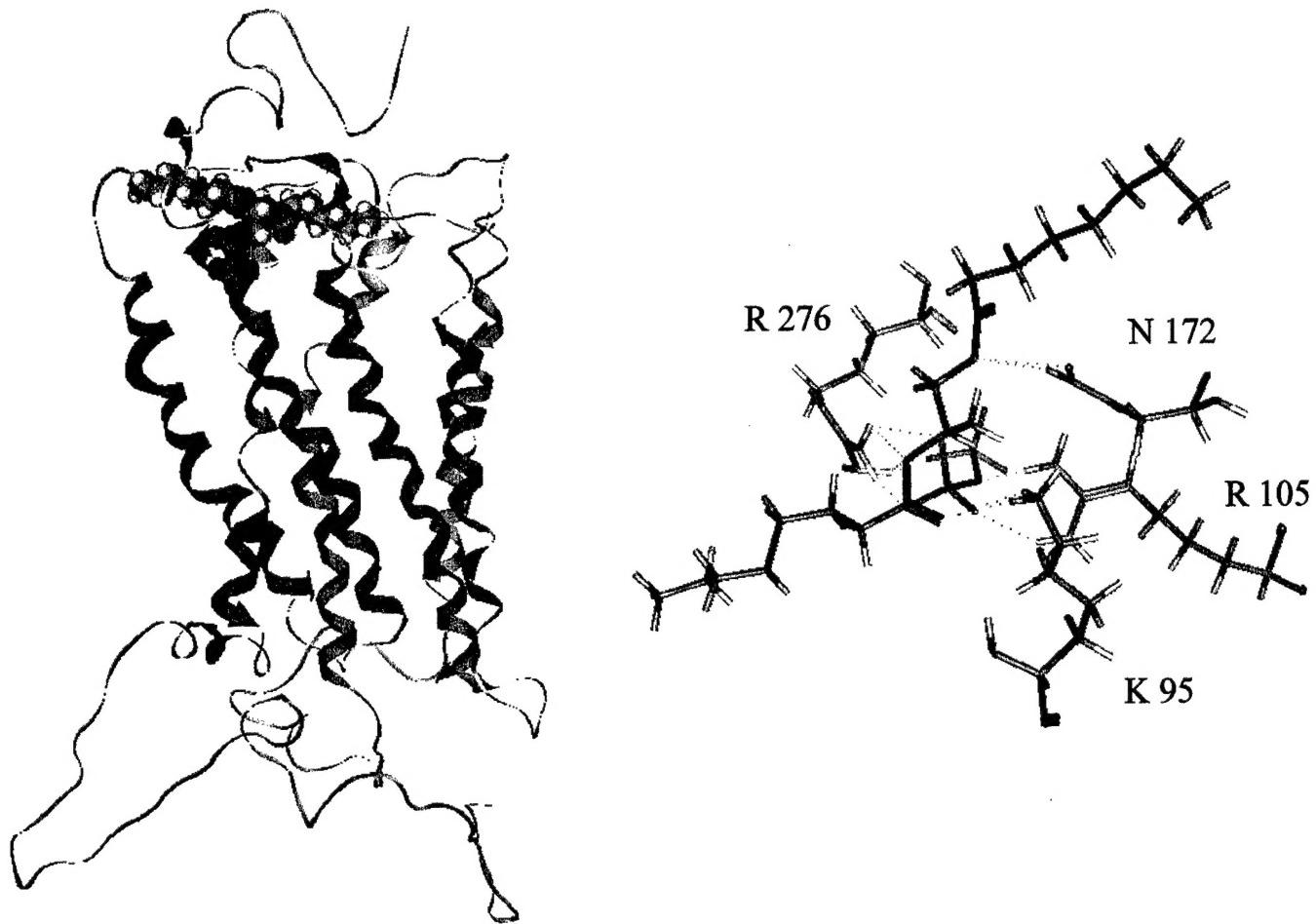
Inactive and Active models of the LPA receptors were developed in our research group

20. An active model of each LPA receptor was developed via homology modeling in MOE

program<sup>21</sup> and was based on the validated model of S1P<sub>1</sub><sup>22</sup>. An inactive model of each LPA receptors was developed by homology modeling in the MOE program and was based on the bovine rhodopsin crystal structure.<sup>23</sup> Autodock 3.0<sup>24,25</sup> was used to assess the docked energies of DGPP (8:0) and FAP (12:0), with the inactive models of LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4, and LPA<sub>3</sub>/EDG7. Both the R and S isomers of LPA (18:1) were docked against active model of LPA<sub>3</sub>/EDG7 to assess the stereochemical preference towards the receptor.

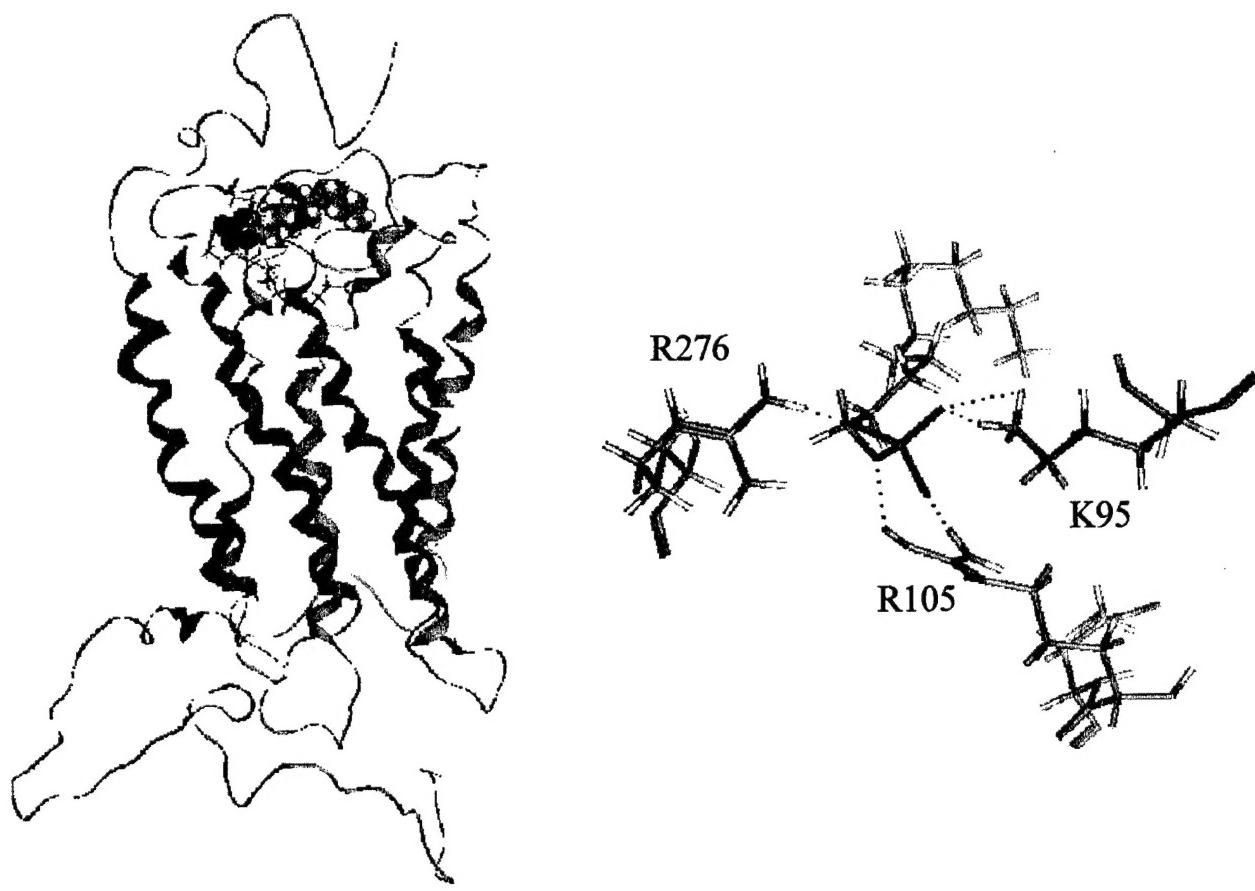
We explored the binding site and assessed the critical amino acid interactions of antagonists and agonists by docking to the inactive and active form of the receptor respectively. The antagonists, DGPP (8:0) and FAP (12:0), were docked against the inactive receptor models. The agonists, R-LPA (18:1) and S-LPA (18:1) were docked against the active receptor models. One hundred complexes of receptor-ligand pair were generated, in order to evaluate the binding region of the ligand. The best ligand-protein complex for each ligand was energy minimized with the MMFF94 forcefield<sup>26</sup> to a RMSG of 0.01 kcal/mol· Å. The ligands were then removed from the minimized receptor-ligand complexes and 100 additional complexes were generated with Autodock in order to re-examine the binding energy after allowing the receptor to acclimatize to the ligand. The docking runs were repeated until the final docked energy converged.

We have docked DGPP (8:0) and FAP (12:0) in our inactive LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> receptor models and our docked energies agree with the observed trend in inhibition constants ( $K_i$ ). The docked antagonist positions overlap the agonist position only at the polar head group, whereas the hydrophobic tails diverge. In the case of the agonist the hydrophobic tail is in the transmembrane (TM) region whereas in the antagonist the hydrophobic tail(s) are in the region just outside the helices. DGPP (8:0) occupies a position just outside the helical bundle towards the extracellular end. The polar head group of DGPP (8:0) forms ion-pairs with K95 (extracellular loop 1), R105 (TM3), and R276 (TM7). The polar head group is also in a position to form a hydrogen bond with N172 (extracellular loop 2), whereas the hydrophobic tail is oriented towards helices IV and V (**Figure 2**). FAP (12:0) also occupies an area outside the helical bundle towards the extracellular end. The phosphate group forms ion-pairs with residues R105 (TM3), R276 (TM7), and K95 (extracellular loop1). The hydrophobic chain is oriented towards helices IV and V (**Figure 3**)



**Figure 2:** On the left is a ribbon model of the inactive form of LPA<sub>3</sub>/EDG7 receptor with DGPP (8:0). On the right is the close-up view of DGPP (8:0) interactions with amino acid residues of LPA<sub>3</sub>/EDG7

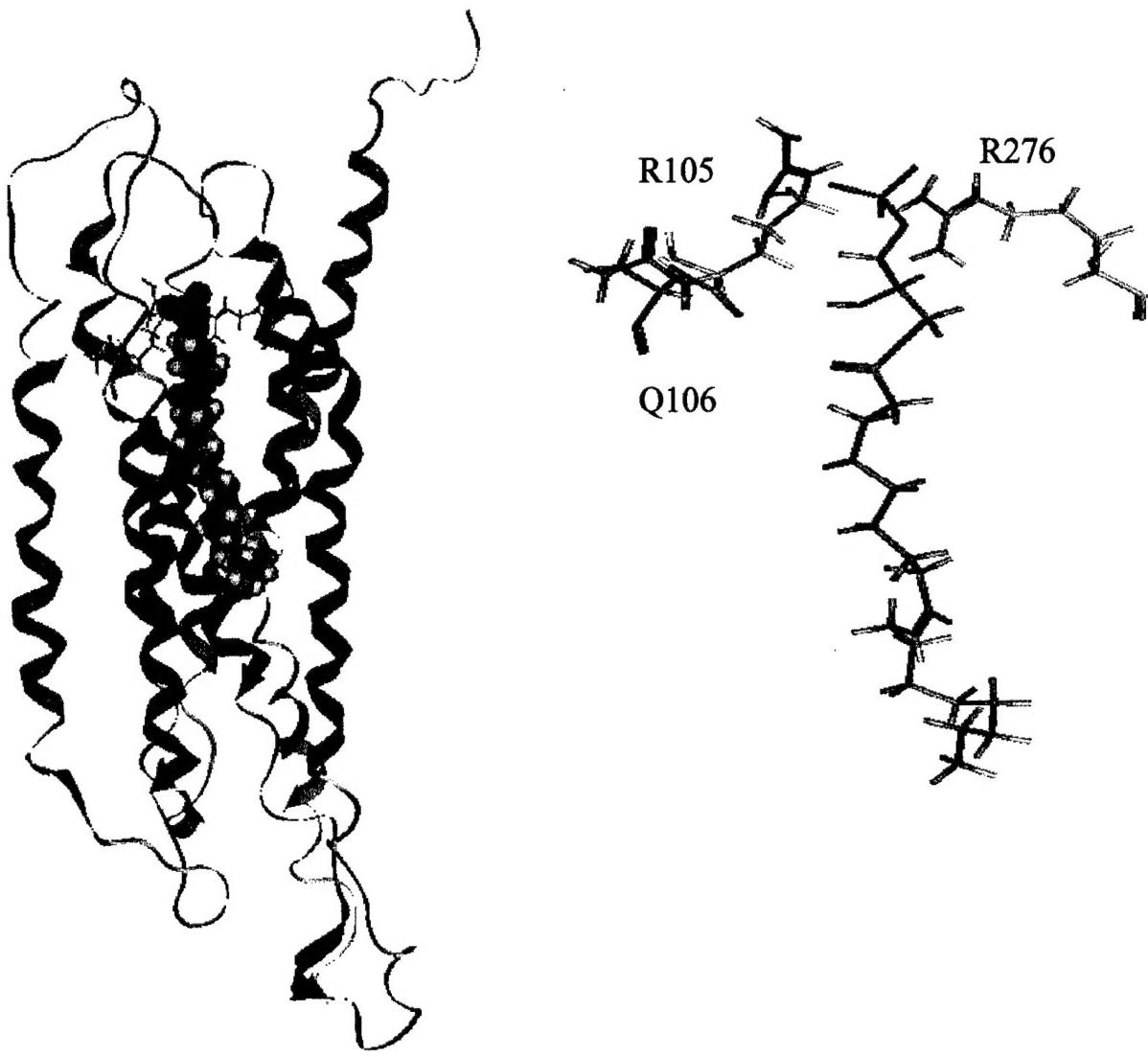
The R and S isomers of LPA (18:1) were docked against the active model of LPA<sub>3</sub> to assess the stereo-selectivity of the receptor. A previous study showed that the R and S isomers of 1-C16-GP (Figure 1) were equally active in mobilizing Ca<sup>2+</sup> in RH7777 cells stably expressing LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub>.<sup>27</sup> Based on our results as shown in Table 1 we do not see any differences in energy with respect to R and S isomer. On assessing the complex of the R isomer of LPA (18:1) with the active form of LPA<sub>3</sub> receptor we observe that the polar phosphate functionality is in close proximity to ion-pair with R105 and R276. We also observe that the hydroxyl group at the *sn*-2 position is in a position to form hydrogen bond with Q106 as shown in figure 4.



**Figure 3:** On the left is a ribbon model of the inactive form of LPA<sub>3</sub>/EDG7 receptor with FAP (12:0). On the right is the close-up view of FAP (12:0) interactions with amino acid residues of LPA<sub>3</sub>/EDG7

LPA (18:1)	Final Docked Energy (kcal/mol)	Intermolecular energy (kcal/mol)	Torsional Free energy (kcal/mol)	Internal energy of the ligand (kcal/mol)
R-isomer	<b>-15.67</b>	-16.64	+6.85	+0.97
S-isomer	<b>-14.78</b>	-18.13	+6.85	+3.35

**Table 1:** Docked energies of R and S isomers of LPA (18:1) against LPA<sub>3</sub>/EDG7



**Figure 4:** On the left is a ribbon model of the active form of LPA<sub>3</sub>/EDG7 receptor with LPA (18:1). On the right is the close-up view of LPA (18:1) interactions with amino acid residues of LPA<sub>3</sub>/EDG7

### Key Research Accomplishments

- Refined models of inactive forms of LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> G-protein coupled receptors
- Refined models of active forms of LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> G-protein coupled receptors
- Identification of a probable site for binding of LPA inhibitors DGPP (8:0) and FAP (12:0) to LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> using Autodock 3.0
- Identification of critical amino acids residues that the above mentioned inhibitors interact with
- Docked R and S isomers of LPA (18:1) to the active form of LPA<sub>3</sub> receptor and our data suggests that there is no significant difference between the binding of the isomers to the receptor

## **Reportable outcomes**

- **Vineet M. Sardar**, Debra L. Bautista, David J. Fischer, Kazuaki Yokoyama, Nora Nusser, Tamas Virag, De-an Wang, Daniel L. Baker, Gabor Tigyi, and Abby L. Parrill. Molecular Basis for Lysophosphatidic Acid Receptor Antagonist Selectivity. *Biochimica et Biophysica Acta* 1582 (2002) 309– 317 (Paper attached in Appendices)
- Tamas Virag, Don B. Elrod, Karoly Liliom, **Vineet M. Sardar**, Abby L. Parrill, Kazuaki Yokoyama, Gangadhar Durgam, Wenlin Deng, Duane D. Miller, and Gabor Tigyi. Fatty Alcohol Phosphates are Subtype-Selective Agonist and Antagonists of LPA Receptors. (*Molecular Pharmacology* 63(5), (2003) **in press**)
- **Vineet M. Sardar**, Tamas Virag, David J. Fischer, Don Elrod, Debra L. Bautista, De-an Wang, Nora Nusser, Kazuaki Yokoyama, Daniel L. Baker, Duane D. Miller, Gabor Tigyi, and Abby L. Parrill. Molecular Modeling of Lysophosphatidic Acid Receptor Antagonists. *ACS 224<sup>th</sup> National Meeting*, Boston, MA, August 18-22, 2002 (**Abstract attached in Appendices MEDI # 79**)

## **Conclusions**

We have refined active and inactive models of LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4, and LPA<sub>3</sub>/EDG7 receptors. A probable binding site just outside the helical domain for LPA<sub>3</sub>/EDG7 inhibitors has been identified. The amino acids that interact with receptor specific inhibitors, DGPP (8:0) and FAP (12:0) have provided us with useful insight. This information can be used to design more potent and selective inhibitors for the prostate-expressed LPA<sub>3</sub>/EDG7 receptor. Rationally designing ligands based on the ligand-protein interactions will aid future attempts to develop receptor-specific compounds, which may be useful in understanding and eventually treating prostate cancer and restricting its invasiveness.

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## Review

## Molecular basis for lysophosphatidic acid receptor antagonist selectivity

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## Abstract

Recent characterization of lysophosphatidic acid (LPA) receptors has made possible studies elucidating the structure–activity relationships (SAR) for agonist activity at individual receptors. Additionally, the availability of these receptors has allowed the identification of antagonists of LPA-induced effects. Two receptor-subtype selective LPA receptor antagonists, one selective for the LPA<sub>1</sub>/EDG2 receptor (a benzyl-4-oxybenzyl *N*-acyl ethanolamide phosphate, NAEPA, derivative) and the other selective for the LPA<sub>3</sub>/EDG7 receptor (diacylglycerol pyrophosphate, DGPP, 8:0), have recently been reported. The receptor SAR for both agonists and antagonists are reviewed, and the molecular basis for the difference between agonism and antagonism as well as for receptor-subtype antagonist selectivity identified by molecular modeling is described. The implications of the newly available receptor-subtype selective antagonists are also discussed. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** G protein-coupled receptor; Molecular modeling; Endothelial differentiation gene receptor; Diacylglycerol pyrophosphate; Homology modeling; Lysophosphatidic acid

## 1. Introduction

During the last decade, enormous progress has been made in identifying and characterizing the cellular receptors specific for the phospholipid growth factor lysophosphatidic acid (LPA) [1–6]. Three of these receptors, LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4, and LPA<sub>3</sub>/EDG7, are members of the endothelial differentiation gene (EDG) family and share 30–35% homology with sphingosine-1-phosphate (S1P) receptors in the same family (S1P<sub>1</sub>/EDG1, S1P<sub>2</sub>/EDG5, S1P<sub>3</sub>/EDG3, S1P<sub>4</sub>/EDG6, S1P<sub>5</sub>/EDG8). Research into the roles of each of these receptors in the biological functions of LPA has consequently developed with groups examining knockout mouse models [7], expression patterns, and functional roles in overexpression systems (for reviews, see Refs. [8–11]). Until very recently, a suite of receptor-selective ligands,

both agonists and antagonists that selectively activate or block specific receptors has been lacking. Here we describe progress that has been made in elucidating the structure–activity relationships (SAR) of these three receptors, as well as in the development of receptor-specific antagonists.

## 2. LPA receptor agonist SAR

Early studies done to elucidate the structural features of LPA that determine its biological activity were performed in many cell lines, as the receptors responsible for the biological effects of LPA were not yet known. Only after the G protein-coupled receptors involved in LPA signaling were cloned and characterized [1,2,4–6] were SAR studies performed in cell lines overexpressing individual receptors. These data can now be pooled and compared to draw conclusions about the receptors responsible for the biological end points observed in various cell lines.

The modifications made to LPA can be discussed based on its structural regions identified as X, R<sup>1</sup>, R<sup>2</sup> and Q in Fig. 1. Of these regions, modifications to X seem to completely eliminate activity. Example modifications that significantly

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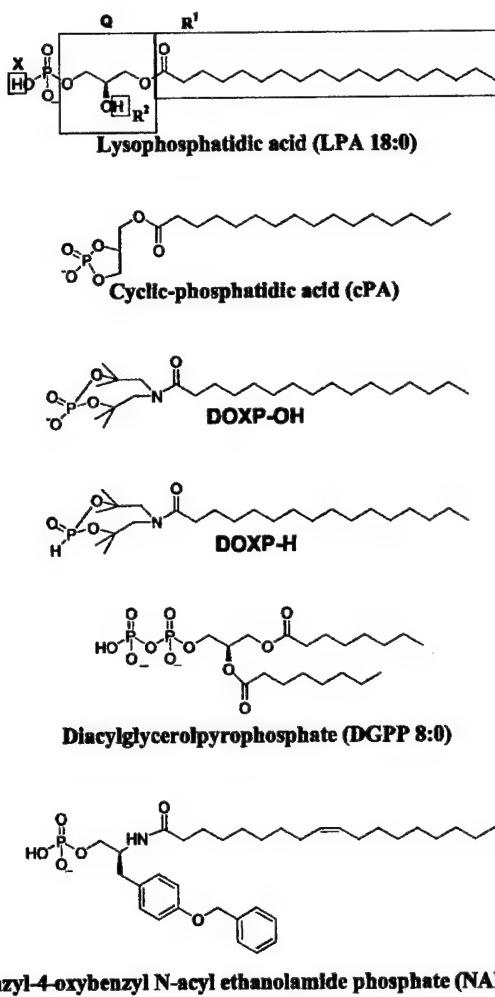


Fig. 1. LPA and analogues utilized to describe the LPA receptor SAR.

alter the size of the headgroup include addition of inositol, ethanolamine, choline and serine, which were all inactive against LPA<sub>1</sub> or LPA<sub>2</sub>-expressing Tag-Jurkat cells [12], LPA<sub>3</sub>-expressing HEK293T cells [2] or LPA<sub>3</sub>-expressing sf9 cells [1]. These same modifications resulted in inactive compounds when the LPA<sub>3</sub> splice variant, HOFNH30, was expressed in RBL-2H3 cells [3]. In whole animal assays in rats, replacement of X with choline, ethanol, propanol, or methanol results in hypotension, rather than the hypertension normally induced by LPA [13]. Several combined modifications in the X and Q regions, including the palmitoyl dioxazaphosphocanes DOXP-H and DOXP-OH (Fig. 1), were tested for their effects on platelet aggregation [14]. Interestingly, DOXP-OH, with its ionizable phosphoryl group, was the only structure to show LPA-like effects. On the other hand, DOXP-H inhibited LPA-stimulated platelet aggregation. Platelets have been shown to express all three of the LPA-selective members of the EDG family (LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4 and LPA<sub>3</sub>/EDG7) [15]. Thus, it is still unclear if DOXP-H might be a general LPA receptor antagonist, or

show some specificity toward one or two LPA receptors. One modification that is well tolerated by all three receptors is cyclization of the phosphate with the 2-hydroxyl of natural LPA to form cyclic phosphatidic acid (cPA, Fig. 1) [1,12]. This modification maintains both the approximate size and the charge of the headgroup. These experimental studies, as well as the recent experimentally validated theoretical model of the LPA<sub>1</sub> complex with LPA [16] all point to a critical interaction between the anionic phosphate group of LPA and its receptors.

The most extensively examined series of LPA modifications have been in the region labeled R<sup>1</sup> in Fig. 1. Assays of Ca<sup>2+</sup>-mobilization in LPA<sub>3</sub>-expressing insect sf9 cells [1,17] and GTP-γ<sup>35</sup>S binding in LPA<sub>3</sub>-expressing HEK293T cells [2] both indicate that unsaturated acyl chains are strongly preferred over saturated acyl chains, whereas LPA<sub>1</sub> and LPA<sub>2</sub> expressed in the same cell types do not show such selectivity. This strong preference of LPA<sub>3</sub> for unsaturated acyl chains as well as the expression of LPA<sub>3</sub> in the heart [1,2] indicates that the cardiovascular effects in rats that are more strongly induced by unsaturated acyl chains [13] are likely mediated by the LPA<sub>3</sub> receptor. All three receptors show considerably decreased or no activity when the acyl chain length is reduced below 16 [2,17]. A combined modification of R<sup>1</sup> and R<sup>2</sup> in the form of 2-acyl LPA has also been tested against LPA<sub>2</sub> and LPA<sub>3</sub>-expressing sf9 cells. LPA<sub>3</sub> shows a strong preference for 2-acyl LPA species over the corresponding 1-acyl LPA species, while LPA<sub>2</sub> shows no such preference [17].

Limited modifications to the region labeled Q in Fig. 1 have been examined in cells overexpressing single LPA receptors. In particular, studies with HEK293T cells expressing either LPA<sub>1</sub>, LPA<sub>2</sub> or LPA<sub>3</sub> demonstrate that the LPA<sub>1</sub> and LPA<sub>2</sub> receptors both accept ethanolamine as a substitute for glycerol, in fact, both are more responsive to the ethanolamine derivatives [2]. LPA<sub>3</sub>, on the other hand, very poorly responds to the ethanolamine compounds.

### 3. LPA receptor antagonists

Within the last year, two separate groups have reported the characterization of selective antagonists for LPA receptors. Fischer et al. reported the testing of a selective and competitive inhibitor of LPA<sub>3</sub>, diacylglycerol pyrophosphate (DGPP) 8:0 (Fig. 1). DGPP 8:0 inhibits LPA-induced Ca<sup>2+</sup> responses in RH7777 cells expressing LPA<sub>1</sub> and LPA<sub>3</sub>, with K<sub>i</sub> values of 6600±680 and 106±28 nM, respectively [18]. Fig. 2 demonstrates this selective inhibition of the LPA-induced Ca<sup>2+</sup> responses in stably transfected RH7777 cells expressing the LPA<sub>3</sub> and LPA<sub>1</sub>, but not in either stably or transiently transfected RH7777 cells expressing the LPA<sub>2</sub> receptor. Heise et al. reported that an N-acyl ethanolamide phosphate (NAEPA) with a benzyl-4-oxybenzyl group at the 2-position of the ethanol backbone (Fig. 1) selectively inhibited LPA-stimulated GTP[γ<sup>35</sup>S] binding in stably transfected LPA<sub>1</sub> and LPA<sub>3</sub>-expressing RH7777 cells, with K<sub>i</sub>

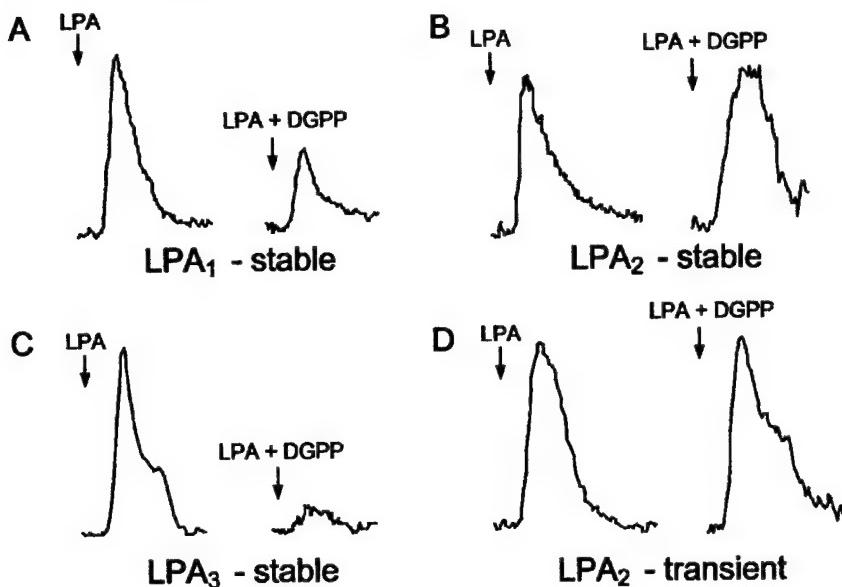


Fig. 2. DGPP 8:0 inhibits the  $\text{Ca}^{2+}$  responses elicited by LPA in LPA<sub>1</sub> and LPA<sub>3</sub>, but not LPA<sub>2</sub> expressing RH7777 cells. RH7777 cells, expressing LPA<sub>1</sub>, LPA<sub>2</sub>, or LPA<sub>3</sub> receptors, were exposed to a mixture of 100 nM (Panels A, C and D) or 1  $\mu\text{M}$  (Panel B) LPA 18:1 and 1  $\mu\text{M}$  (Panels A, C and D) or 10  $\mu\text{M}$  (Panel B) DGPP 8:0. Control cells were exposed to 100 nM (Panels A, C and D) or 1  $\mu\text{M}$  (Panel B) LPA 18:1. Representative  $\text{Ca}^{2+}$  responses are shown for stable LPA<sub>1</sub> (Panel A), LPA<sub>2</sub> (Panel B), and LPA<sub>3</sub> (Panel C) expressing cells, or cells transiently expressing LPA<sub>2</sub> (Panel D). Stable LPA<sub>2</sub> cells had a higher EC<sub>50</sub> for LPA as compared to cells transiently expressing the same construct. Nevertheless, the high 10  $\mu\text{M}$  concentration of DGPP 8:0 was still ineffective in inhibiting the response.

values of 137 and 428 nM, respectively [19]. Since the assays used to characterize these antagonists were not the same, the  $K_i$  values cannot be compared to determine the relative efficacy of the antagonists. It is interesting to note, however, that neither antagonist shows activity against LPA<sub>2</sub>/EDG4, and that they show opposite preferences for LPA<sub>1</sub> and LPA<sub>3</sub>.

These antagonist structures share a significant feature with the known SAR for the LPA receptors. The polar headgroup of both structures is anionic, either phosphate or pyrophosphate. However, both of these structures contain two hydrophobic chains, a considerable change from the typical agonist at these receptors. One hydrophobic chain in each structure is relatively short, 8 carbons in the case of DGPP and approximately 10 carbons long in the case of the benzyl-4-oxybenzyl group. The structures differ considerably in the composition of the second lipid chain, which includes 8 carbons in the case of DGPP 8:0 but 18 carbons in the case of the NAEPA analog. The presence of one short hydrophobic chain seems to be important as neither DGPP 18:0 nor phosphatidic acid 18:0 were antagonists at the LPA receptors [18].

#### 4. Receptor structure and amino acids critical for ligand recognition

The intriguing similarities and differences between the known agonists and antagonists of the LPA receptors led us to apply molecular modeling techniques to explore the basis for both the observed antagonist selectivity for LPA<sub>1</sub> and LPA<sub>3</sub>, and for agonism versus antagonism.

#### 4.1. LPA receptor modeling

Models of the LPA receptors were developed by homology to our previously validated S1P<sub>1</sub> model [20]. The validated S1P<sub>1</sub> model is consistent with experimental studies exploring the impact of site-directed mutations on receptor activation, and thus these models, referred to as the 'A' models, represent the active conformation of these receptors. Since antagonists should have equal affinity for the inactive conformation, we also developed models of each receptor based on the recently published bovine rhodopsin crystal structure, which was characterized in its dark, inactive state, termed the 'I' models [21]. These 'I' models, since they are based on the only experimental structure of a G protein-coupled receptor, are likely to accurately represent regions outside the transmembrane domain. This is not the case for models based on the S1P<sub>1</sub> model, which was based on a template structure that did not have atomic coordinates outside the transmembrane domain. The validation of the S1P<sub>1</sub> model additionally includes only critical amino acid contacts with S1P, all of which are within the transmembrane domains. All receptor models were developed and optimized according to the procedures we have applied to the development of our validated S1P<sub>1</sub>/EDG1 and LPA<sub>1</sub>/EDG2 models [16,20].

Ballesteros and Weinstein [22] developed a unique nomenclature for identifying residues that are in corresponding locations in different GPCRs. This nomenclature uses the most conserved residue of a helix and identifies it with the helix number and then the extension of 50 (example: W4.50 is the most conserved residue in the fourth helix).

Fig. 3 identifies each helical reference position on an alignment of bovine rhodopsin and the LPA receptor subfamily. All residues in the transmembrane domain can be numbered relative to the helical reference position. This nomenclature is particularly useful for identifying amino acids that are in the same position within the transmembrane domains of different receptors and so will be used throughout this text in order to better compare the LPA<sub>1</sub>, LPA<sub>2</sub> and LPA<sub>3</sub> complexes.

#### 4.2. Docking studies

Docking computations were performed to determine interactions between ligand and receptor. The resulting complexes were then used to determine whether differences

exist between the members of the LPA receptor family that explain the experimentally observed differences in affinity for DGPP. Two different docking methods were utilized to fully explore the interactions between DGPP and the LPA receptors and to quantitatively correlate computed docking energies with the experimentally observed trend in selectivity. The Tabu algorithm [23] in the MOE computer program (version 2001.01, Chemical Computing Group, Montreal) with random ligand starting positions, was first used to generate up to 50 complexes for the appropriate receptor models and either DGPP or 18:1 LPA. The resulting putative complexes were evaluated as in our previous studies for the presence of electrostatic interactions between the receptor and ligand [20,24,25]. Autodock 3.0 [26] was additionally used to generate 100 complexes of DGPP with the 'I' models

Rhod	.....	MNGTEGPNFYVPPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIML
LPA <sub>1</sub>	MAAISTSIPIV1SQPQFTAMNEPQCFY	-NESIAFFYNRSGKHLATEWNTV--SKLVMLGLITVCIF
LPA <sub>2</sub>	.....	MV1MGQCYY-NET1GFFYNNSGKELSSHWRPK--DVVVVALGLTVSVL
LPA <sub>3</sub>	.....	MNECHYD-KHMDFFYNRNSNTDTVDDWTGTK--LV1VLCVGTFCLF
80		
Rhod	51 GFPINFLTLVYTVQHKKLRTPLNY1	LLNLAVADLFMVFGGF <del>TTTLY</del> TSLHGKFVFGPTGCNLEGF
LPA <sub>1</sub>	63 IMLANLLVMVAIYVNRRFHFP1	YYLMANLAA <del>ADFF</del> AGLAYFYL <del>M</del> FNTGPNT-[RRLTVSTWLLRQG
LPA <sub>2</sub>	46 VLLTNLLVIAAIASNRRFHQP1	YLLGNLAA <del>DL</del> FAGVAYLFLM[H]TPR <del>T</del> -ARLSLEGWFLRQG
LPA <sub>3</sub>	44 IFFSNSLVIAAVIKNRKFHF <del>P</del> YYLLANLAA <del>ADFF</del> A <del>G</del> IAYVFLMFNTGPVS-[K]LT <del>V</del> NRWFLRQG	82 82 82 82
116		
Rhod	116 FATLGGEIALW <del>S</del> LVVLA <del>I</del> ERYVVCKPM <del>S</del> NFRFGENHA	MGVAF <del>T</del> W <del>M</del> ALACA <del>APPL</del> VGWSRYI
LPA <sub>1</sub>	127 LDTS <del>L</del> TASVANLLAIA <del>I</del> ERHITVFRMQLHTRMSNRRVVV	IIVV <del>W</del> TM <del>A</del> VMGA <del>I</del> PSVGNNCIC-
LPA <sub>2</sub>	110 LLDT <del>S</del> LTASVATLLAIAVERHRSVMAVQLHSRLPRGRVVML	IVGVWWAALGLG <del>L</del> PAHSMHCLC-
LPA <sub>3</sub>	108 LDSS <del>L</del> TASLTNLLVIAVERHMS	IMRMRVHSNLTKRVTLL <del>I</del> LLW <del>A</del> <del>I</del> FMGA <del>V</del> PTLGNNCLC-
181		
Rhod	181 EG <del>M</del> QCSCGIDYYTPHEETNNESFV1YM <del>F</del> VVF <del>H</del> 1	PLIVIFFCYGQLVFTVKEAAA---QQQESAT
LPA <sub>1</sub>	191 D <del>E</del> NC <del>S</del> NMAPLYSDSYLVFWA <del>I</del> FN <del>L</del> VT <del>V</del> V <del>M</del> V <del>V</del> YAH <del>I</del> FG	-YVRQRTMRMSRHSSGPRR
LPA <sub>2</sub>	174 ALDRCSRMAPLLSRSYLA <del>V</del> W <del>A</del> LLSLLV <del>F</del> LM <del>V</del> AVYTRIFF	-YVRRRVQRMAEHVSCHPR
LPA <sub>3</sub>	172 NISACSSLAPIYSRSYLVFW <del>T</del> V <del>S</del> NLMA <del>F</del> IMVVVYL <del>I</del> YV	-YVKRKT <del>N</del> V <del>L</del> SPHTSGSIS
243		
Rhod	243 TQKAKEVTRMV1	IMVIAFLICWLPYAGVAFY1
LPA <sub>1</sub>	249 NRDTMMSLLKTVV1	VLGAF1CWT <del>P</del> GLV <del>L</del> LLD-VCCPQCDV <del>L</del> AYE[ <del>K</del> ] <del>F</del> LLLA <del>E</del> FNSAMNP1
LPA <sub>2</sub>	232 YRETTLSLVKTVV1	LGAFVV <del>C</del> WT <del>P</del> QVV <del>L</del> LDGLGCESC <del>N</del> V <del>L</del> AVE <del>K</del> YF <del>L</del> LLAEANS <del>L</del> VNAAVYS
LPA <sub>3</sub>	230 RRRTPMKLMKTVMTVLGAFVV <del>C</del> WT <del>P</del> GLV <del>V</del> LLDG <del>L</del> NCRQCGVQHV <del>K</del> W <del>F</del> LLALLNSVVNP1	11YS 750
308		
Rhod	308 MNKQFRNCMV <del>T</del> LCGK	-NPLGDDEASTTVSKTETSQ--VAPA
LPA <sub>1</sub>	313 YRDKE <del>M</del> SATFRQ1LCCQR	-SENPTGP--TEGSDRSASSL--NHT1LAGVHSNDHSVV
LPA <sub>2</sub>	297 CRDAEMRRTF <del>R</del> LLCCAC	-LRQSTRESVHYTSSAQGGASTR1MLPENGHPLMT <del>PP</del> F <del>S</del> YLELQRY
LPA <sub>3</sub>	295 YKDEDMYGT <del>M</del> KK1CCFSQENPERRPSRIPSTVLSRS <del>D</del> TG	--SQYIEDS1SQGAVCNKSTS

LPA<sub>2</sub> 360 AASNKSTAPDDLWVLLAQPNQ

Fig. 3. Aligned sequences for the LPA family and rhodopsin. From top, bovine rhodopsin, human LPA<sub>1</sub>, human LPA<sub>2</sub> and human LPA<sub>3</sub>. Helical reference positions are indicated above the appropriate position, as are specific positions mentioned in the text. Transmembrane helices including extensions beyond the membrane observed in the bovine rhodopsin crystal structure are underlined. Boxes highlight residues that docking studies indicate are important in binding the pyrophosphate group of DGPP 8:0.

of each LPA receptor in order to more completely explore the binding site found by the Tabu algorithm in the vicinity of the extracellular loops, as well as to provide a more quantitative reflection of the relative affinity of DGPP for each receptor.

The best complex for each receptor was then energy minimized with the MMFF94 forcefield to a gradient of 0.01 kcal/mol Å to allow the receptor to conform to the presence of DGPP. DGPP was then removed and 100 additional com-

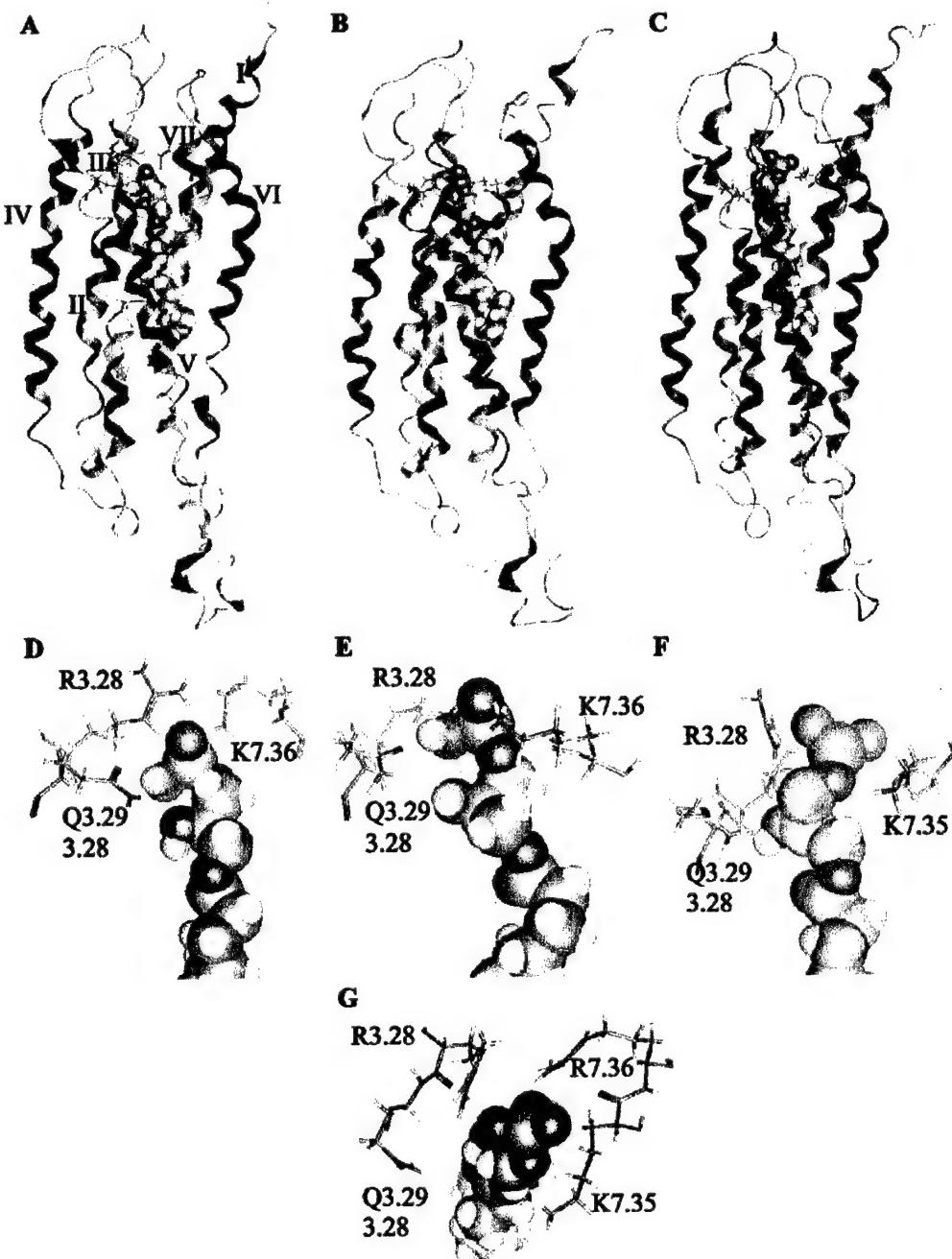


Fig. 4. Models of LPA receptors with 18:1 LPA. Proteins are shown as ribbon models with 18:1 LPA as a space-filling model. Residues forming key interactions with the polar headgroup of 18:1 LPA are shown as stick models. The transmembrane domains are numbered in Panel A using Roman numerals, the orientation of these domains in Panels B and C is the same. LPA<sub>1</sub> (Panel A), LPA<sub>2</sub> (Panel B) and LPA<sub>3</sub> (Panel C) complexes with 18:1 LPA viewed from within the cell membrane. Close-up of interactions between LPA<sub>1</sub> (Panel D), LPA<sub>2</sub> (Panel E) and LPA<sub>3</sub> (Panel F) and the polar headgroup of 18:1 LPA viewed from the same perspective as in Panels A, B and C. (Panel G) Close-up of interactions between LPA<sub>3</sub> and the polar headgroup of 18:1 LPA viewed from the extracellular surface (rotated approximately 90° from the orientation shown in Panel F).

plexes were generated with Autodock 3.0 in order to reevaluate the binding energy after allowing the receptor model to adapt to the presence of the ligand. If the position found prior to minimization was not found in the 100 additional complexes generated, the docking run was repeated. Once a docking run succeeded in finding the pre-minimized position, the complex with the lowest docked energy (whether the ligand was in the pre-minimized position or not) was selected as the best complex.

#### 4.3. 18:1 LPA complexes

Using 18:1 LPA and the 'A' models of LPA<sub>1</sub>, LPA<sub>2</sub> and LPA<sub>3</sub>, the complexes generated during the docking studies

were evaluated to determine the amino acids involved in agonist binding. On examination of the LPA receptor family, a clear and consistent picture of LPA binding emerged. Three residues are predicted to be necessary for binding LPA: R3.28, Q3.29 and K/R7.36, Fig. 4. An additional residue, K7.35, may be important for LPA binding to LPA<sub>3</sub>. In LPA<sub>1</sub>, mutation of Q3.29 (Q125) to A completely abolished binding of LPA [16]. This prediction is also consistent with the S1P docking studies with S1P<sub>1</sub> [20,24,25]. These studies experimentally validate the importance in S1P<sub>1</sub> of R3.28, E3.29 and R7.34 for binding of S1P. In the alignment of the LPA and S1P receptor families, positions 3.28 and 3.29 are conserved across the two families, but the position of the third residue is not as strictly conserved in the S1P receptor family.

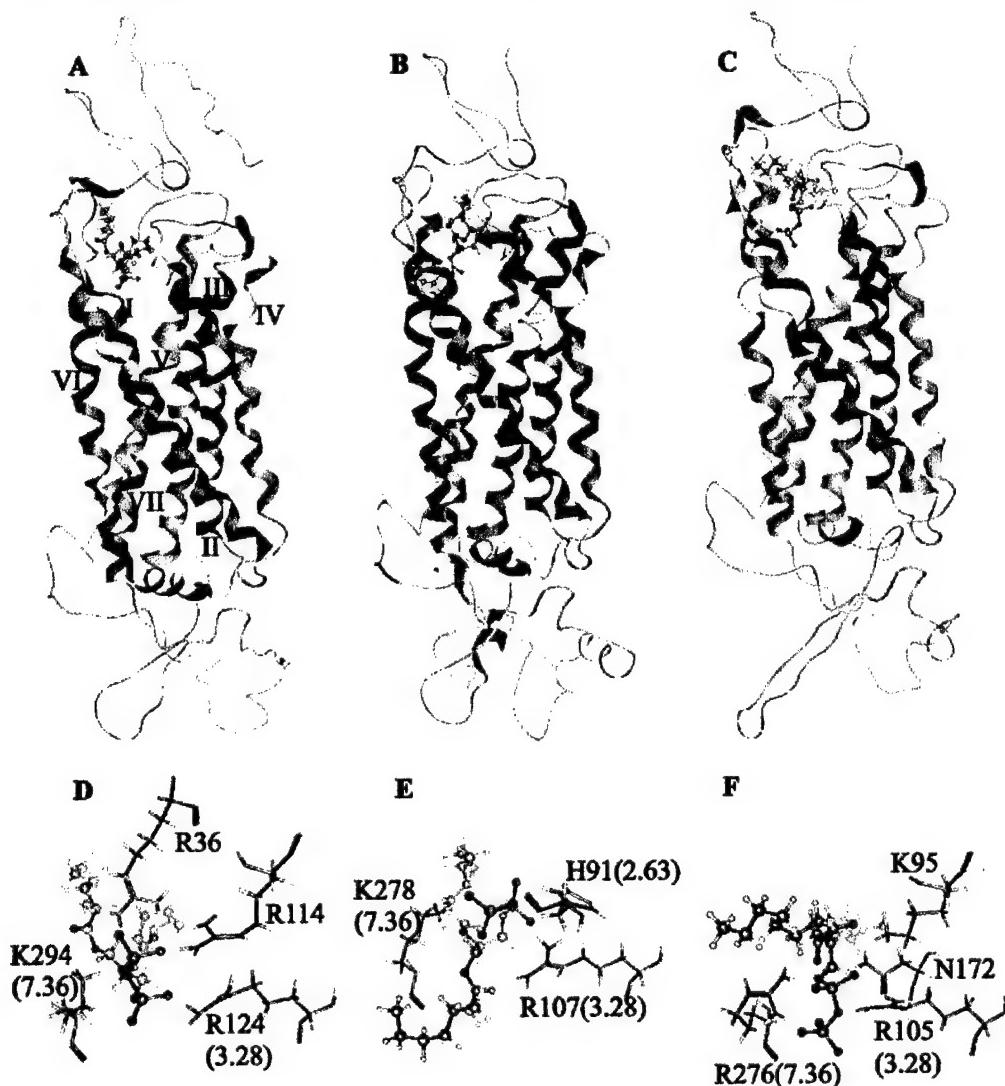


Fig. 5. Models of LPA receptors with 8:0 DGPP. Proteins are shown as ribbon models with 8:0 DGPP as a ball and stick model. Residues forming key interactions with the polar headgroup of 8:0 DGPP are shown as stick models in Panels D–F. The transmembrane domains are numbered using Roman numerals in Panel A, the orientation of these domains is the same in Panels B and C. LPA<sub>1</sub> (Panel A), LPA<sub>2</sub> (Panel B) and LPA<sub>3</sub> (Panel C) complexes with 8:0 DGPP viewed from within the cell membrane. Close-up of interactions between LPA<sub>1</sub> (Panel D), LPA<sub>2</sub> (Panel E) and LPA<sub>3</sub> (Panel F) and the polar headgroup of 8:0 DGPP viewed from the same perspective as in Panels A, B and C.

#### 4.4. DGPP 8:0 complexes

The complexes generated by the MOE program were evaluated for interactions between the receptor and the ligand based on the number of hydrogen bonds. The complex with the most hydrogen bonds was used to determine the optimal region of the receptor structure to more thoroughly explore with the Autodock program. Docked energies from the Autodock program were utilized to select the best complex in order to compare interactions across the LPA receptor family.

Evaluation of the complexes between DGPP and the 'I' model of the DGPP-selective LPA<sub>3</sub> receptor produced multiple entries that occupied the same volume at the extracellular end of the transmembrane helical bundle, yet within the extracellular loops. The complex with the lowest docked energy (-12.4 kcal/mol) is shown in Fig. 5. The polar head group forms hydrogen bonds with residues K95 (extracellular loop 1), R105 (3.28), N172 (extracellular loop 2) and R276 (7.36). The complex of LPA and LPA<sub>3</sub> indicates that R3.28 and R7.36 are necessary for agonist binding and their involvement in binding both the agonist and antagonist is consistent with the experimental result that DGPP is a competitive antagonist of LPA action at LPA<sub>3</sub>. The hydro-

phobic tails of DGPP interact entirely with residues at the extracellular end of the transmembrane domains and with residues in the extracellular loops. One hydrophobic chain is oriented toward transmembrane helices IV and V. The second hydrophobic chain is oriented toward transmembrane helix I (Fig. 6).

Complexes of the LPA<sub>1</sub> receptor, model 'I', with DGPP showed geometric features similar to the best complex of the higher affinity LPA<sub>3</sub> receptor complex, but with a poorer docked energy (-11.9 kcal/mol). Fig. 5 shows the best complex between DGPP and LPA<sub>1</sub>. The polar headgroup of DGPP forms hydrogen bonds with R36 (amino-terminus), R114 (extracellular loop 1), R124 (3.28) and K294 (7.36). The complex of LPA and LPA<sub>1</sub> predicts that R3.28 and K7.36 are necessary for agonist binding and their interaction with the antagonist is consistent with the experimental result that DGPP competitively antagonizes LPA action at LPA<sub>1</sub>. The hydrophobic tails of DGPP interact entirely with residues at the extracellular end of the transmembrane domains and with residues in the extracellular loops. The hydrophobic tails in this complex are both oriented in the direction of helices IV–VI (Fig. 6).

The best DGPP complex generated with the DGPP-unresponsive LPA<sub>2</sub> has a significantly higher docked energy

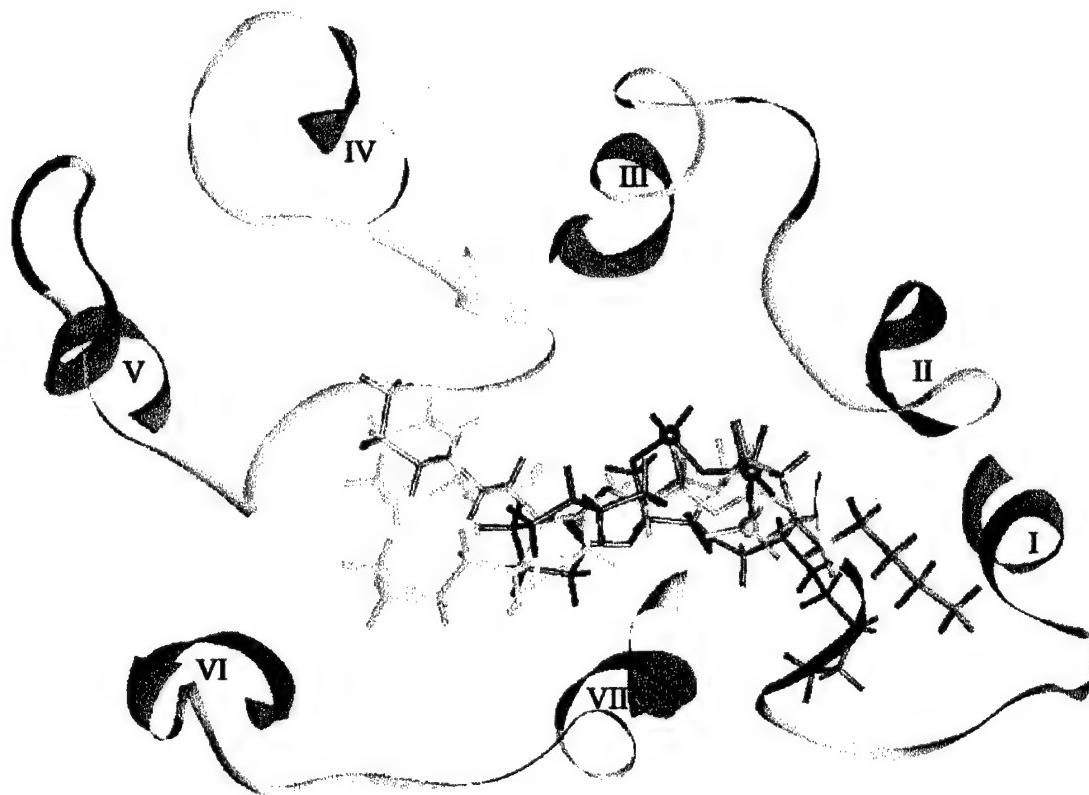


Fig. 6. Overlay of DGPP complexes with LPA<sub>1</sub>, LPA<sub>2</sub> and LPA<sub>3</sub>. For clarity, ribbons are shown only for the extracellular loops and the first helical turn into the membrane for LPA<sub>2</sub>, ribbons for the other two receptors are analogous. The transmembrane helical domains are numbered with Roman numerals. DGPP structures are shown as stick models with the phosphorous atoms as larger balls. The green DGPP structure is the optimal position in LPA<sub>1</sub>, blue shows the optimal position in LPA<sub>2</sub> and orange shows the optimal position in LPA<sub>3</sub>.

(−9.4 kcal/mol) than the DGPP-selective LPA<sub>3</sub> complex (−12.4 kcal/mol) or the moderately responsive LPA<sub>1</sub> complex (−11.9 kcal/mol). These predicted differences agree with the experimental observation that LPA action at LPA<sub>2</sub> is not inhibited by DGPP while DGPP demonstrates a 106 nM  $K_i$  at LPA<sub>3</sub> and a 6600 nM  $K_i$  at LPA<sub>1</sub> [18]. Only three residues were found to form hydrogen bonds with DGPP, H91 (2.63), R107 (3.28) and K278 (7.36) (Fig. 5). Two of these, R3.28 and K7.36 are predicted to interact with LPA. The hydrophobic chains of DGPP in this complex are oriented toward transmembrane helices I and VII (Fig. 6).

### 5. Implications of selective LPA receptor antagonists

The LPA<sub>1</sub>-selective antagonist, benzyl-4-oxybenzyl NAEPA, and the LPA<sub>3</sub>-selective antagonist, DGPP 8:0, are invaluable pharmacological tools for researchers examining the signaling pathways of the LPA receptors in numerous cell types. The endogenous co-expression of multiple LPA-responsive receptors in a wide variety of cells has previously been a significant challenge to researchers wishing to examine the biology of LPA without the complication of potential artifacts due to receptor overexpression.

The modeling results presented herein indicate that the molecular basis for the observed competitive antagonism by these compounds is due to a common interaction site for the anionic headgroup of both agonists and antagonists. The anionic phosphate group of both DGPP and LPA is anchored to the receptor through interactions with a common set of cationic amino acids in transmembrane helices III and VII. However, a very different interaction exists between DGPP and the extracellular loops. Preliminary docking studies with benzyl-4-oxybenzyl NAEPA indicate that this antagonist interacts with the extracellular loops in a manner similar to DGPP. These results suggest that antagonists can be developed that completely diverge from the phospholipid structures explored thus far against these receptors.

Finally, LPA receptor antagonists can now be explored as potential therapeutic lead compounds. The importance of LPA in angiogenesis [27,28], inflammation [29], cancer invasiveness [30] and atherosclerosis [31,32] demonstrates but a few of the potential processes that might be targeted by LPA receptor agonists and antagonists.

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**ABSTRACTS**

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**W. J. Greenlee, Program Chair**

79.

MOLECULAR MODELING OF LYSOPHOSPHATIDIC ACID RECEPTOR

ANTAGONISTS. *Vineet M. Sardar<sup>1</sup>, Tamas Virag<sup>2</sup>, David J. Fischer<sup>2</sup>, Don Elrod<sup>3</sup>, Debra L. Bautista<sup>1</sup>, De-an Wang<sup>2</sup>, Nora Nusser<sup>2</sup>, Kazuaki Yokoyama<sup>2</sup>, Daniel L. Baker<sup>2</sup>, Duane D. Miller<sup>3</sup>, Gabor Tigyi<sup>2</sup>, and Abby L. Parrill<sup>1</sup>.* (1) Department of Chemistry and Computational Research on Materials Institute, University of Memphis, 3744 Walker Suite # 213, Memphis, TN 38152-6060, Fax: 901-678-3447, vsardar@memphis.edu, (2) Department of Physiology and Biophysics, University of Tennessee, (3) Department of Pharmaceutical Sciences, University of Tennessee

Lysophosphatidic acid (LPA) elicits a variety of responses including mitogenesis, cytoskeletal changes, activation of  $\text{Ca}^{2+}$  transients, and effects on apoptosis. These responses are elicited via LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4, and LPA<sub>3</sub>/EDG7 G protein-coupled receptors. These receptors are members of the endothelial differentiation gene family. In order to understand the physiological significance of LPA highly selective antagonists are necessary. Recently, dioctyl glycerol pyrophosphate (DGPP) and fatty alkyl phosphate (FAP) were shown to be potent and selective antagonists towards LPA<sub>3</sub> receptor. We have docked DGPP and FAP in our LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> receptor models and our docked energies agree with the observed trend in inhibition constants ( $K_i$ ). The docked positions of the antagonist relative to the agonist overlap in the position of the polar head group, but diverge in the favored position of the hydrophobic tail(s).